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Gross anatomy and scanning electron microscopic study of the tongue of Egyptian Dabb lizard (*Uromastyx aegyptia*) in relation to feeding habits

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Abstract

Introduction: The tongue is considered a key innovation in the evolution of a terrestrial lifestyle.

Methods: The adult animals were sacrificed with sharp razor blade. The heads were separated to remove out tongue from its root after separation of upper and lower jaws.

Results: The tongue of *Uromastyx aegyptia* is divided into fore-tongue with its anterior tip, mid and hind tongue. The anterior region of the fore-tongue is bifurcated. The lingual papillae are posteriorly imbricated and separated by wide trenches. The epithelial cells are provided by tiny microvilli. In the mid-tongue, the papillae are not imbricated and have strong rhombus-shaped with narrow trenches. The microvilli of the surface epithelium have reticular pattern and attached with each other by microridges. The papillae of the hind-tongue have a rectangular shape with deep serrated ends. The imbricated part in the anterior portion of each papilla is provided with numerous taste buds. The striated muscle fibers are distributed in the lamina propria of the lingual papillae and the tongue connective tissue.

Conclusion: The distribution and orientation of the muscle fibers prove that the tongue is able to catch the prey by biforked tip and facilitate the swallowing behavior.

Keywords: Uromastyx, reptilia, lingual apparatus, microscopy, morphology.

Introduction

The tongue is often considered a key innovation in the evolution of a terrestrial lifestyle as it allows animals to transport food particles through the oral cavity (Iwasaki, 2002; Herrel et al., 2005). Also, the tongue has been adapted for a wide diversity of functions such as prey capture, drinking, breathing, and defensive behaviors (Bels et al., 1994; Schwenk, 1995 and Darwish, 2012). Moreover, there are fairly strong correlations between tongue anatomy and its functional roles (e.g., food transport and manipulation), and the environmental conditions in

which animals use their tongues or hyobranchial system (i.e., water vs. air) (McClung and Goldberg, 2000; Schwenk, 2000; Iwasaki, 2002; Herrel *et al.*, 2005; Darwish, 2012).

The shape and structure of the tongue differ significantly among animal species, reflecting the various functions of each respective tongue (**Iwasaki**, **2002 and Santos** *et al.*, **2011**). In the anatomy of the tongue, three parts may be distinguished: the apex, the body and the root (**Dehkordi** *et al.*, **2010**, **Al-Zahaby**

and Elsheikh, 2014 and Al-Zahaby, 2016). On the dorsum of tongue surface, there are various kinds of lingual papillae including filiform, fungiform, circumvallate and foliate papillae, each having different morphological structure and shape.

Distribution of these lingual papillae has been considered to be related to species eating habits and vocalization (**Park and Lee, 2009**). All the papillae are covered by stratified squamous epithelium that differs by the thickness and keratinization only (**Iwasaki and Miyata, 1985** and **Wassif, 2001**).

The aim of the present work is to study the morphology and histology of the tongue of Egyptian dabb lizard, *Uromastyx aegyptia* (Forskal, 1775), by the aid of light and scanning electron microscopy, to reveal the form and function of the tongue with comment on its significance.

Materials and Methods

Four adult specimens of Egyptian Dabb lizard (Uromastyx aegyptia) (Family: Agamidae - Reptile) were collected from Abou -Rawash area of Egypt. The animals were transferred alive to the laboratory where they left for 24hrs in the laboratory conditions .Animals were sacrificed and their heads were separated to remove out tongues from root with the aid of sharp razor blade after separation of upper and lower jaw and. Gross-morphology photographs were captured by using Samsung 12 mega pixel digital camera for subsequent investigations For light microscopic investigation, small parts of tongue (apex, body and root) were fixed in 10% neutral formalin, dehydrated in ascending series of alcohols, cleared in xylene and processed for paraffin blocks. Longitudinal and transverse paraffin sections of 4-5µm thickness of the three parts of the tongue were cut and stained with Hematoxylin and Eosin (H & E) staining (**Drury and** Walington 1980). However, for the SEM studies, Animal's tongues were fixed in 10% formalin solution. After complete fixation, specimens were washed twice in the phosphate buffer solution to remove excess of fixative and then Post-fixed in 1% osmium tetroxide osmium-phosphate buffer (PH 7.8) at 4°C,

consequently rinsed twice in the same phosphate buffer solution. Specimens were then infiltrated with iso-amyl acetate, dried by the critical point drier in Hatachi Critical Point Drier (HCPD), mounted on aluminum stubs, sputtered with gold in Joel fine coat Ion sputter. After then tongues were investigated and photographed under JEOL scanning electron microscopy (JSM-5300) at an accelerating voltage of 15kv scanning electron in the Regional Centre of Mycology at Al-Azhar University, Cairo, Egypt.

Results

Gross Morphology:

The tongue apparatus of the Egyptian dabb lizard, *Uromastyxaegyptia* 1 (Forskal, 1775), is like of other Reptile can be differentiated into t anterior free portion and posterior laryngeal area (Figs. 1, 2 & 3). The free portion is triangular in shape with shallow bifurcated pointed apex and broad base posteriorly. It is occupied the greater part of the buccal-cavity's floor and has two well developed posterior limbs between which lies the laryngeal area (Fig.3). The free portion is of about 1.7 cm in length and 5 mm in width and distinguished into three parts, apex (A), body (B) and root (R) (Figs.1-3). The tongue is attached to the floor of the mouth through, skin fold lying under the posterior end of the free portion.

The roof of the oral cavity of this Egyptian dabb lizard is characterized by opened fenestra exochoanalis bounded by the maxilla laterally, the vomer medially, the vomer-maxilla contact anteriorly, and the palatine posteriorly. The anterio-lateral margin of the vomer shows a lateral embayment, the incisura Jacobsoni, within which the opening of Jacobson's organ lies. The internal naris (choana) opens at the posterior end of the fenestra exochoanalis and bounded by the palatine. The anterior end of the palatine is characterized by a variably developed but generally shallow and short choanal groove. The margin of the upper jaw bears a single row of pointed cone-shaped teeth whereas the lower jaw bears two rows of pointed cone- shaped teeth (Fig.2).



Fig.1:A photo of dorsal surface of the tongue of *U.aegyptia*, showing the free portion which differentiated into; shallow bifurcated tip(BFT), lingual apex (A), mid tongue (body) B and hind tongue (root) R, pre-glottal region (PG), laryngeal mound(LM),groove (G) and glottis (GL).

Fig.2: A photograph of buccal cavity (roof and floor) of *U.aegyptia*, showing the tongue occupied a greater part of floor of the lower jaw and the upper jaw with its related structures, internal naris(IN), pointed cone shaped teeth(CT), premaxilla (Pm), maxilla (M), vomer (V), palatine (Pa) and choanal groove(Chg).

Fig.3: Scanning Electro-micrograph of dorsal surface of the tongue of *U.aegyptia*, showing shallow bifurcated tip (BFT), lingual apex (A), body (B), root (R) and groove (G). The preglottal region (PR), laryngeal mound (LM) and large glottis(GL),Scale bar one mm.

Scanning Electron Microscopic Observations:

By SEM it was found that, the morphology of the Egyptian dabb's tongue is triangular in shape with a distinct lingual shallow bifurcated apex. The free portion of the tongue is divided into the three portions described above; apex (A) (fore-tongue), body (B) (mid-tongue) and root (R) (hind-tongue) as shown in figure (3).

Generally, the tongue's dorsum of the present 1 lizard is papillosed with widely distributed flattened filiform papillae (FP)of different shapes. These papillae increased in size and attained a scaly appearance towards the posterior end of the tongue (Fig.4).With higher magnification, these flattened filiform papillae showed plumose cells, plumose papillae (PL)with thickening cell margin (CL) as showed in(Fig.5). Moreover, some of circumvallate papillae(CVP) can be observed in-between irregular shape filiform papillae. These circumvallate papillae areof rounded shape and pointed center, each is surrounded by small pointed filiform ones (Fig.6).

At the lateral side of the fore-tongue, lot of filiform papillae (FP) appeared lined up next to each other and most of them have a pointed end (Fig.7). With higher magnification, it was found that, these papillae bear microridges and embracing some taste buds (TB), one or more per each papilla (Fig.8).



Fig.4: Scanning Electro-micrograph of the lingual apex of *U.aegyptia*, showing flattened filiform papillae (FFP) attained a scaly appearance, Scale bar 20µm.

Fig.5: Scanning Electro-micrograph of higher magnification showing, plumose cells (PL), thickening borders of the cells (arrow), Scale bar 2μ m.

Fig.6: Scanning Electro-micrograph of the dorsal surface of the posterior portion of the lingual apex of *U.aegyptia* showing irregular shapes of filiform papillae (FP) and circumvallate papillae (CVP), Scale bar 50 μ m.

Fig.7: Scanning Electro-micrograph of the lateral side of the lingual apex of *U.aegyptia*, showing irregular shapes of filiform papillae, most of them are terminated with a pointed ends (arrow), Scale bar 100 μ m.

However, the mid-tongue (body) of *U. aegyptia* bears also filiform papillae (FP) but of irregular shapes (Fig.9). In the lateral side of this tongue region, the apical surfaces of these filiform papillae are covered with keratinized sheets (K)as illustrated in(Fig.10). Nonetheless, the dorsum of the hind tongue's segment (root) bears compact pointed conical filiform papillae (CFP). These papillae are over lapped on each other and can be also called canine-like papillae (Fig.11). At higher magnification ,plumose cells are also pointed out on these papillae(Fig.12).



Fig.8: High magnification of Scanning Electro-micrographof filiform papilla, showing microridges (MR), a taste bud (TB) (one or more per papilla) and intracellular borders (arrow), Scale bar one μ m.

Fig.9: Scanning Electro-micrograph of the dorsal surface of the lingual mid-tongue (body) of *U.aegyptia* showing different forms of filiform papillae (FP), Scale bar 50 μ m.

Fig. 10: Scanning Electron-micrograph of the dorsal surface of lateral side of lingual body of *U. aegyptia* showing, the apical surfaces of the filiform papillae(FP) covered with keratinized sheets (K), Scale bar 20 μ m.

Fig.11: Scanning Electro-micrograph showing, filiform papillae (FP), conical filiform papillae (CFP) appeared overlapped on each other, Scale bar 20 μ m.

At the most posterior portion of the hind-tongue, openings of taste buds and glands are observed (Fig. 14).

On the other hand, the laryngeal area is differentiated into; preglottal region (PG) anteriorly and laryngeal mound (LM) posteriorly (Fig.13). The preglottal region is of a semicircular shape and limited anteriorly by the posterior border of the free portion of the tongue and posteriorly with the large glottis (GL) (Fig.13). Numerous gland openings (GO) are distributed on the whole surface of the laryngeal area (Fig. 15).



Fig.12: Scanning Electro-micrograph showing plumose cells (PL) are scattered on the canine – like papillae, Scale bar 20 μ m.

Fig.13: Scanning Electro-micrograph of the dorsal surface of the laryngeal area of *U.aegyptia*, showing preglottal region(PG), laryngeal mound(LM) surrounding the large glottis (GL), Scale bar one mm.

Fig.14: Scanning Electro-micrograph of the dorsal surface of the posterior portion of the hind tongue of *U. aegyptia* showing, opening of taste buds(TB) and glandular opening (GO) in between microridges (MR) of the scattered filiform papillae(FP), Scale bar 100 μ m.

Fig.15: Scanning electron micrograph of the dorsal surface of the laryngeal area of *U.aegyptia*, showing the openings of glands of this area (G0) . X:8,500

Histological Observations

The mucosa of the bifurcated lingual apex of *U.aegyptia* is covered with a relatively thick stratified squamous epithelium (SE) which appeared partially keratinized. The dorsal surface of that region contained large number of flattened filiform papillae(FP), some are characterized by their pointed ends (Figs. 16&17). Moreover, filamentous filiform papillae are also observed on the dorsal surface (Fig.18).Some taste buds are distributed too in the

epithelium of these lingual papillae (Fig. 19). Avery few circumvallate papillae (CVP)are also observed among filiform papillae in the lingual apex area . They are characterized by abroad surface with depressions in its margins and covered with a thick layer of stratified squamous epithelium (Fig.20). The dorsal epithelium of the circumvallate papillae is manifest by minute bumps or projections as shown in (Fig. 20). All of these papillae have dense connective tissue mainly in the central region (Fig.16).



Fig.16: Photomicrograph of a vertical section through the lingual apex of the free portion of the tongue of *U.aegyptia*, showing conical filiform papillae (CFP), Keratin (K), Taste bud(TB) and large acini of the lingual glands (LG).(H&E), Scale bar 800µm.

Fig. 17: High magnification of fig. 16, showing conicalfiliform papillae (CFP) with its pointed edge, Keratin (K) and connective tissue core (CT). (H&E), Scale bar 330 μ m.

Fig.18: Photomicrograph of a vertical section through the fore-tongue (lingual apex) of *U.aegyptia*, showing filamentous filiform papillae (arrow) and wide lumen of the acini of lingual gland (LG). (H&E), Scale bar 800 μ m. **Fig.19:** Photomicrograph of a vertical section through the fore-tongue (lingual apex) of *U.aegyptia*, showing taste buds (TB) (one or more taste bud for each papilla). (H&E), Scale bar 80 μ m.

The sub-mucosa has large acini of lingual glands, these acini are with wide lumens occupied by an eosinophilic homogeneous secretion (Figs. 16&18).

Similarly, the mucosa of the dorsal surface of the midtongue (body) is covered also by keratinized stratified squamous epithelium. Filiform papillae of different shape are extensively distributed all over the dorsum of this region. They are of either tall, slender or conical shape (Fig.21).

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Moreover, taste buds (TP) are widely spread among these filiform papillae (Fig. 22).The corium is formed of a well distinct lamina propria (LP) with a thick, dense, irregular layer of connective tissue ,positioned just under the epithelium. Skeletal muscle fibers (MF) originating from the intrinsic muscle bundles are also observed passing through the lamina propria (Figs. 20). Many lingual glands of the alveolar type occupy the lamina propria of the dorsal surface of this mid tongue region (Fig. 21). These glands are opened on the surface with a long narrow neck (Fig.23).



Fig.20: Photomicrograph of a vertical section through the fore-tongue (lingual apex) of *U.aegyptia*, showing circumvallate papilla(CVP) with some projections (arrow) is found among filiform papillae(FP), connective tissue core (CT) and striated muscle fibers (MF).(H&E), Scale bar 800µm.

Fig.21: Photomicrograph of a vertical section in the mid-tongue (body) region of *U.aegyptia*, showing different keratinized (K) filiform papillae (FP) of different shape; tallfiliform papillae (TFP) and conical filiform papillae (CFP). Acini of lingual glands (LG) with its eosinophilic secretion are also detected.(H&E), Scale bar 800 μ m.

Fig.22: Photomicrograph of a vertical section through the mid-tongue (body) of *U.aegyptia*, showing the taste buds (TB) are distributed among the epithelia of the filiform papillae (FP) and connective tissue core (CT) also appeared. (H&E), Scale bar 80 μ m.

The mucosa of the hind-tongue (root) of *U.aegyptia* is covered mainly with partially keratinized conical filiform papillae (CFP) in addition to few of fungiform ones(Fig. 24).The same figure also showed branched alveolar lingual glands (LG) well distributed elsewhere in the corium (Fig. 24).The conical papillae are with curved pointed tips oriented posteriorly toward the tongue root, however its core are composed of collagenous connective tissue fibers (Figs.25). Taste buds are observed among filiform papillae in the posterior third of the hind-tongue of this studied reptilian animal species (Fig.26).



Fig.23: Photomicrograph of a vertical section through the mid-tongue (body) of *U.aegyptia*, showing the long narrow neck (arrow) of the lingual gland (LG) and its opening in the lingual epithelium (GO) and lamina propria (LMP). (H&E), Scale bar 80 μ m.

Fig.24: Photomicrograph of vertical section through the hind-tongue of *U.aegyptia* showing fungiform papillae (FUP), conical filiform papillae (CFP), enormous branched lingua lglands (LG)with their openings (GO) and longitudinal muscles(LM). (H&E), Scale bar 330 μ m.

Fig.25: Photomicrograph of vertica lsection through the hind- tongue of *U.aegyptia*, showing pointed conical filiform papillae (CFP) covered by keratin(K), connective tissue core (CT) and lingual gland (LG). (H&E), Scale bar 80 μ m. **Fig.26:** Photomicrograph of vertical section through the posterior third of hind- tongue of *U.aegyptia*, showing the taste buds (TB) are observed in filiform papillae (FP) and connective tissue core (CT).(H&E), Scale bar 80 μ m.

Discussion

The vertebrate tongue plays a very important role in food intake, its structural variations appear to represent adaptation to the current environmental conditions of each respective habitat (**Iwasaki, 2002**) and so reptilian tongue is characterized by morphological and functional variations among different species. According to (Schwenk, 1985, 1986&1989; Smith, 1988 and Toubeau *et al.*, 1994) lizards showed different morphological and histological structures of the tongue among species in different habitats. The tongue's morphology of the Egyptian dab lizard (*Uromastyx aegyptia*) is characterized by the presence of many types of lingual papillae that distributed on the categorized lingual

region: fore, mid and hind-tongue except its smooth shallow bifurcated distal tip. This is correlated with their habitat and consequently type of food as also proved by **Parchami** *et al.* (2010) indicated a close correlation of the structural morphology of the tongue with the type and method of food intake.

The anterior shallow bifurcated tip of the tongue of *Uromastyx aegyptia* is also presented in all squamate reptiles except legless lizards. However, a deep bifurcation was reported in snakes and varanids (Oelofsen and Van Den Heever,1979;Smith and Mackay,1990; Attia *et al.*,1998;de Groot *et al.*, 2004 and El-Sayyad *et al.*, 2011). The slightly bifurcation of tongue was also documented in Gekkonidae (Mohammed,1991) but it is absent in Arabian Toad head Agama (Ferguson,1981), Sphenodon (Schwenk, 1986) and turtles (Iwasaki,1992).

The present observations revealed that, the tongue of *Uromastyx aegyptia* is triangular in shape, it have broad base posteriorly and pointed bifurcated tip. The tongue exhibited the presence of grooves parallel to tongue apex which facilitated flexion. Fixed tongue and macroscopic structure accommodated the animal for mode of feeding. A similar result has been reported by (**El-Sayyad** *et al.*, **2011**) in *Tarentola annularis* and *Crocodylus niloticus*. Three areas are observed on the dorsal lingual epithelium of *Uromastyx aegyptia*: fore tongue and its anterior tip, mid and hind-tongue. This result was also demonstrated by (Wassif,2002) who worked on *Chalcideso cellatus* (**Delheusy** *et al.*,**1994**) worked on the iguanid lizard *Oplurus cuvieri* and by (**Herrel** *et al.*,**2005**).

The present SEM examination demonstrated that, the dorsal region of the tongue of Uromastyx aegyptia is covered with many types of lingual papillae that showed clear differences between the fore, mid and the most posterior part of the tongue. These differences appeared in shape, size, number and distribution as also showed in lacertid lizard. Lacertaviridis (Bels et al., 1993). These papillae are increased in size and attained a scale-like appearance towards the posterior end of the tongue. This was discussed by Sarhan and Hussein (2013) since, they suggested that, the orientation of the imbricated papillae either to the posterior as in Chalcides ocellatus or anterior as in Chalcides episodes may be related to the drinking behavior of lizards. The same authors declared that, in Chalcides ocellatus, the posteriorly imbricated papillae serve in sipping water, while the imbrications towards the anterior direction indicates that the lizard may suck food solutions rather

than drinking it and used to eject the prey's remnant after sucking their squeezer.

The flattened filiform papillae, plumose cells and thickening cell margin are widely distributed all over the apical lingual surface of the Egyptian dabb lizard's tongue. These plumose cells are designated as plumose papillae according to **Rabinowitz and Tandler (1986)**. They reported that, the plumose papillae are composed of connective tissue core covered by stratified squamous epithelium surface from where numerous elongated cells radiated. These plumose papillae are assumed to play an important role in the interlocking of the tongue onto prey surface **(Schwenk, 2000)**.

Moreover, some of circumvallate and fungiform papillae which are observed in-between the irregular shape filiform papillae, are also presented in the tongue's root of many vertebrates. The first, circumvallate papillae, were detected in the dwarf armadillo, *Zaedyus pichiy* (Ciuccio *et al.*, 2008) and Long-eared hedgehog, *Hemiechinus auritus*(Taha, 2013) but the second one, fungiform papillae, were showed on the tongue surface of Iraqi sheep by Husseinand AL-Asadi (2010)as well as of *Bufo regularis* (Elsheikh *et al.*, 2013).

The microridges widely distributed over the surface epithelia of the Egyptian dabb lizard's tongue, may be helpful for receiving and collecting chemical molecules during tongue flicking as demonstrated by (**Toubeau** *et al.*, **1994**). Otherwise, these microridges was commonly associated with mucous secreting epithelia. These micro-structures serve to hold in place a residual layer of mucus, hich acts as a lubricant and facilitate movement of material over a cell surface (**Whitear,1990** and **Iwasaki,1992**).Similarly, **Al-Zahaby and Elsheikh (2014)** and **Al-Zahaby (2016)** described microridges as structures that increase the adhesion of mucus to the epithelium.

Light microscopic observations of the Egyptian dabb lizard revealed that ,its lingual surface exhibited the presence of different forms of filiform papillae including ;circumvallate, fungiform, con-shaped filiform papillae. The latter two papillae presented on the hind-tongue surface were also detected on tongue surface of American alligator (**Shimada** *et al.*, **1990**)and Nile crocodile (**Putterill and Soley, 2003**). Otherwise, keratinization characterize of stratified squamous epithelium the lingual epithelial surface of *Uromastyx aegyptia* was also recognized in many reptiles such as lizard, *Eumeces schneideri*, Scincidae (Wassif, 2001) and *Eublepharis macularius*, Gekkonidae (Jamniczky *et al.*, 2009) even in some birds like as common kingfisher (Al-Zahaby and Elsheikh, 2014)and cattle egret (Al-Zahaby, 2016). However non keratinization of the lingual epithelium was evidencedin many vertebrates like as lacertid lizards *,Dromaiusnovae hollandiae*, (Herrel *et al.*,2005), Emus *,Dromaius novaehollandiae* (Crole and Soley, 2009) and hooded crow, *Corvus corone cornix* (Elsheikh and Al-Zahaby, 2014).

Lamina propria in the three parts of tongue of the present Uromastyx aegyptia which is formed of dense connective tissue is rich with blood vessels and striated muscle fibers appeared in different arrangement and density .The distribution and orientation of the muscle fibers prove that the tongue is able to catch the preys by its bifurcated tip. and facilitate the swallowing behavior .The extensive development of the lingual muscles reflects the essential role of tongue in catching and swallowing behavior. These muscles documented in all vertebrates especially in reptilian species with its organization help tongue movement to performmany functions (Cooper, 1998; Herrel et al., 1998, 2001 & 2005 and Jamniczkyet al., 2009).

Otherwise, in the present work, the presence of lingual glands of the branched alveolar type especially in the hind-tongue segment was evidenced also in many different Reptilian species as *Sphenodon* (Schwenk, 1986), Turtles (Winokur, 1988), iguanid lizard (Delheusy *et al.*,1994), golden lizard(Wassifand El-Hawari, 1998) and *Acanthodactylus* lizard (Mohammed,1992). The secretion of large amounts of mucus acts as a lubricant that facilitates food movements, transport and swallowing (Wassif, 2002;Mohammed *et al.*,1998).

Taste buds in the lingual epithelium which observed in the present study are distributed all over the dorsal surface of the tongue .These taste buds are also observed iniguanids (Delheusy et al., 1994), lizards (Schwenk,1985), Tuatara (Schwenk,1986), turtles (Winokur,1988) and alligators(Ferguson,1981). They are of testing the palatability during prey's capture (Delheusy et al., 1994). They are stimulated by the molecules transferred during tongue flicking (Toubeau et al.,1994). These previous findings present observations in Uromastyx support the aegyptia.

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